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التأثير الوقائي لأوراق الجنكة وبذور السينا على الفئران المصابة بإرتفاع الكوليسترول

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Abstract

This study looked at how *Ginkgo biloba* leaves (GB) and *Cassia tora* seeds affected hypercholesterolemic rats lipid profiles for eight weeks. Thirty six adult male rats were divided into two main groups. The first main group (six rats) were fed a regular diet and used (-ve control group). The second main group of 30 rats, fed on a high-cholesterol diet to induce hypercholesterolemia for 7 weeks, then was divided into five subgroups as follows: Subgroup (1) was fed a high-cholesterol diet and used as a positive control group. Subgroup (2 and 3) were given high-cholesterol diets that included supplements of 5% and 10% Gb leaves powder, respectively. Subgroup (4 and 5) were given high-cholesterol diets that included supplements of 5% and 10% powder from *C. tora* seeds, respectively. According to the obtained findings, liver enzymes, kidney functions and lipid profile were found to be significantly decreased (P≤0.05) while, HDL-C was noticeably increased by Gb leaves or *C. tora* seeds at the tested levels as compared to the positive control group. Furthermore, significantly increased in antioxidant enzymes (SOD and CAT) while, a decrease in MDA by Gb leaves or *C. tora* seeds supplementation at the tested levels in hypercholesterolemic rats. In conclusion that Gb leaves or *C. tora* seeds significantly reduced cholesterol levels while also improved liver enzymes, kidney functions and antioxidant enzyme. Therefore, Gb leaves or *C. tora* seeds may be recommended for hypercholesterolemic patients.

Keywords:

Ginkgo biloba leaves, *Cassia tora* seeds, Hypocholesteremia, lipid profile, antioxidant status.

التأثير الوقائي لأوراق الجنكة وبذور السينا على الفئران المصابة بارتفاع الكوليسترول

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مستخلص البحث:

اجريت هذه الدراسة لمعرفة تأثير اوراق الجنكة وبذور السينا على مستويات الدهون فى الفئران التي تعاني من فرط الكوليسترول لمدة 8 أسابيع. تم تقسيم ستة وثلاثين فأرا من ذكور الفئران إلى مجموعتين رئيسيتين. المجموعة الرئيسية الأولى (6 فئران) تم تغذيتها على النظام الغذائي الأساسي (كمجموعة ضابطة سالبة). المجموعة الرئيسية الثانية (30 فأرًا) تم تغذيتها على نظام غذائي مرتفع الكوليسترول لإحداث فرط كوليسترول بالدم لمدة سبع أسابيع ثم تم تقسيمهم الي خمس مجموعات على النحو التالي: المجموعة الفرعية (1) تم تغذيتها على نظام غذائي مرتفع الكوليسترول (كمجموعة ضابطة موجبة). المجموعة الفرعية (2 و3) تم تغذيتهم على نظام غذائي مرتفع الكوليسترول مضاف إليه 5% و10% من مسحوق اوراق الجنكة على التوالي. المجموعة الفرعية (4 و5) تم تغذيتهم على نظام غذائي مرتفع الكوليسترول مضاف إليه 5 % و10% من مسحوق بذور السينا على التوالي. أظهرت النتائج حدوث تحسنا في انزيمات الكبد ووظائف الكلى ، كما لُوحظ ارتفاع الكوليسترول الجيد باستخدام اوراق الجنكة او بذور السينا عند المستويات المختبرة مقارنة بالمجموعة الضابطة الموجبة. علاوة على ذلك ، زادت بشكل ملحوظ الإنزيمات المضادة للأكسدة (SOD، CAT) بينما انخفض مستوى MDA عند إضافة اوراق الجنكة وبذور السينا عند المستويات المختبرة للفئران المصابة بإرتفاع كوليسترول الدم. يمكن الاستنتاج أن اوراق الجنكة وبذور السينا لها تأثير ملحوظ في تقليل مستوى الكوليسترول بالدم وتحسين انزيمات الكبد ووظائف الكلى والإنزيمات المضادة للأكسدة .لذلك يمكن ان يُوصى باستخدام اوراق الجنكة وبذور السينا لمرضى ارتفاع الكوليسترول بالدم.

الكلمات المفتاحية:

اوراق الجنكة ، بذور السينا ، فرط كوليسترول الدم ، صورة دهون الدم ، الحالة المضادة للأكسدة

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Introduction

By 2030, it is predicted that hypercholesterolemia, a kind of hyperlipidemia, will be responsible for 40% of deaths **(Jeong *et al*.,2019)**. Epidemiological studies have shown that unhealthy eating habits, such as consuming too many foods high in cholesterol and saturated fats, are strongly linked to the prevalence of hypercholesterolemia, which increases the risk of atherosclerosis **(Stewart *et al*., 2020)**. Synthetic hypercholesterolemic medications are becoming less and less used due to their, due to its negative side effects and the establishment of treatment resistance. So, since plants constitute an essential source of chemical compounds, there has been an increase in the usage of medicinal herbs **(Atanasov *et al*., 2015)**.

The only extant species of the Ginkgophyta division, *Ginkgo biloba* (Gb), is one of the most widely used medicinal plants for the treatment of a variety of illnesses **(Shu *et al*., 2018)**. The name "Ginkgo" is sometimes known as maidenhair fern. Due to the similarity in shape and veining of its leaves to those of that plant **(Singh *et al*., 2019)**. Gb is used all over the world in the prevention and treatment of various cardiovascular diseases (CVDs), both as a pharmaceutical and a food supplement **(Silva & Martins, 2023)**. In addition, it has been shown that Gb could rescue renal injury in adriamycin-induced hyperlipidemic nephrotoxicity **(Li *et al*., 2017)**. GB leaves contain a wide variety of biologically active chemicals such as flavonol, glycosides (kaempferol, quercetin, and isorhamnetin), diterpenes, and sesquiterpenes **(Gafner, 2022)**. Numerous studies have shown that Gb and its active ingredients have antioxidant, anti-inflammatory, antiplatelet, and metabolic-regulating properties, all of which contribute to its anti-atherosclerotic actions **(Chen *et al.*, 2019 and Bian *et al*., 2022)**.

Cassia tora is a species of wild plant in the *Leguminosae* family endemic to Central America, its common name is sickle senna. In East Asia, folk medicine has traditionally made extensive use of whole plants, seeds, leaves, and roots. The seeds are edible, and when they are roasted, they can be used in place of coffee **(Mostafa *et al*., 2021)**. *C. tora* seeds contain bioactive compounds, particularly phenolics, flavonoids, anthocyanins, and antioxidants **(Islam *et al*., 2023)**. The seed or leaves of C. tora are beneficial for anti-hyperlipidemia, and anti-obesity, due to their pharmacological effects, which include anti-inflammation, antidiabetic, antihepatotoxic and anti-oxidant properties (**Hwang *et al*., 2022 and Ko *et al*., 2020)**. Therefore, the aim of this study was to examine the cholesterol-lowering effects of ginkgo leaves or cassia tora seeds on hypercholesterolemic rats.

Materials and Methods

- **Materials**
  Rats: Thirty six (36) adult male albino Sprague Dawley rats weighed around (170 ±10 g) were obtained from Helwan Farm, Cairo, Egypt.

- **Chemicals:** Casein, cellulose vitamins, minerals, cholesterol and bile salts were obtained from Sigma Chemical, Cairo, Egypt.

- **Kits** were acquired for Biodiagnostic Reagents from Alkan Company, Dokki, Cairo, Egypt.

- **Plant:** *Ginkgo biloba* leaves and *C. tora* seeds were purchased the Agricultural Research Centre, Cairo, Egypt.

- **Methods**

Preparation of *Ginkgo biloba* leaves and *Cassia tora* seeds powder: Using an electric blender, the plant material was ground into a fine powder after being shade-dried for four days. Using an electric stainless steel mill, *Ginkgo biloba* leaves or *Cassia tora* seeds were crushed into a fine powder and sieved through an 80-mesh screen before being kept in the plastic container.

Inducing hypercholesterolemia in rats: The hypercholesterolemic diet, which was modified slightly from **Reeves *et al*., (1993)**, contained casein (14%), cellulose (5%), vitamin mixture (1%), sucrose (10%), mineral mixture (3.5%), choline bitartrate (0.25%), maize oil (4%), l-cystine (0.18), and the remaining ingredients were starch plus cholesterol (1%), and bile salt (0.25%) **(Pandya *et al*., 2006)**.

Biological study: After a period of acclimation, the rats were divided into two main groups: the first main group (n=6) had a standard diet as a healthy control group (-ve) during the experimental period, while the second main group (n=30) received a high cholesterol diet. After that, the rats were divided into 5 Subgroups, each including six rats: Subgroups (1): Rats were administered a high cholesterol diet (+ ve control group). Subgroups (2 and 3) were given high-cholesterol diets that included supplements of 5% and 10% Gb leaves powder, respectively. Subgroups (4 and 5) were given high-cholesterol diets that included supplements of 5% and 10% powder from C. tora seeds, respectively. Rats were starved overnight before being sacrificed at the end of the study period (8 weeks), and their blood was then taken and centrifuged. Separated serum was kept at -20^{0} C until analysis.

Biological Evaluation: feed intake (FI) and body weight gain percent (BWG %) were calculated according to (Chapman *et al*., 1959) using the following equation:

$$BWG\% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

Chemical analysis:

Serum Lipid Profile: According to **Allain (1974), Fassati & Prencipe (1982)**, and **Albers *et al*., (1983)**, the serum total cholesterol (TC), triglycerides (TG), and cholesterol contents of high-density lipoprotein (HDL-c) were measured, respectively. low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated according to **(Friedewald *et al*., 1972)**.

$$LDL\text{-}c = TC - [HDL\text{-}c + (TG/5)] \qquad VLDL\text{-}c = TG/5$$

Liver Function: Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to **Bergmeyer *et al*. (1978)**, serum alkaline phosphates (ALP) were measured **(Belfield & Goldberg 1971)**.

Kidney Function: According to **Kaplan (1984), Patton and Crouch (1977),** and **Murray (1984)**.Serum urea, uric acid, and creatinine were determined, respectively.

Oxidant and antioxidant enzymes: Following **Draper and Hadley (1990)** methodology, the plasma level of malondialdehyde (MDA) was calculated to measure lipid peroxidation. Superoxide dismutase (SOD) activity was evaluated by **Spitz and Oberley, (1989)**. Catalase (CAT) was measured by **Aebi, (1984)**.

Statistical Analysis: The SPSS programme was used to analyse the results that were collected. Results from the ANOVA test were compared between groups, and a significance level of $P<0.05$ was considered **(Snedecor and Cochron, 1989)**.

Results and Discussion

The outcome of *Ginkgo biloba* leaves and *Cassia tora* seeds on feed intake (FI) and body weight gain % (BWG) was shown in Table (1). Results showed non-significant changes in FI (g/day/rat) in rats fed on basal diet negative control (–ve), in comparison to hypercholesterolemic rats fed on the same diet. On the other hand, hypercholesterolemic groups, which were treated with Gb leaves or C. tora seeds at levels (5 and 10%) , showed non-significant changes in the mean values of FI, as compared to negative control (–ve) and hypercholesterolemic rats (+ve group).

Furthermore, the mean value of BWG% in hypercholesterolemic groups positive control (+ve) increased more than that of negative control (–ve). While, all treated groups with supplementation with Gb leaves or C. tora seeds at levels (5 and 10%) showed a significant decrease $p \leq 0.05$ in BWG%, comparison to the control (+ve) group.

Moreover, the highest decrease in BWG% in the experimental group was recorded for the group which treated with C. tora seeds and *Ginkgo biloba* leaves at 10%. The high fat diet (HFD) proved successful in causing obesity; rats on the HFD had significantly higher body weights. It is well known that high-fat diets increase body weight and visceral fat deposition, and such findings have previously been published **(Banin *et al*., 2014 and Kubant *et al*., 2015)**. Similary, **Hirata *et al*., (2019)** observed prior to GbE intervention, we noticed a greater relative FI in rats fed an HFD diet for the entire two month obesity-induction period.

In tune with the results of the present study, **Banin *et al*., (2021)** and **Hirata *et al*., (2019)** observed Gb, positive changes in body weight decrease and a decrease in fat cells (adipocytes). **Naseem *et al*., (2016)** observed a considerable loss in BW in diabetic rats, but therapy with GBE brought the BW back to normal although being less reduced. Our findings for the decrease in BW by Gb supplementation in HFD rats are consistent with earlier findings. **Khattab, (2012)** observed that the GBE treatment marginally increased food intake and BWG%. Concerning the effect of *Cassia tora* seeds **Ko *et al*., (2020)** found *C. tora* seeds significant reduction in the BW in HFD rats. **Tzeng *et al*., (2013)** showed that Eight weeks after Rats receiving the *Cassia seed* ethanol extract (CSEE) (300 mg/kg/day) therapy had significantly lower BWG% than rats receiving HFD.

Table (1): Effect of *Ginkgo biloba* leaves and *Cassia tora* seeds on feed intake (FI) and body weight gain % (BWG) in hypercholesterolemic rats

Parameters / Groups	FI g/day/each rat	BWG%
Control (-ve)	17.500 [a]± 1.00	20.100 [d]± 1.431
Control (+ve) Hch diet	17.55 [a]± 0.707	32.698 [a]± 1.727
***Ginkgo biloba* leaves 5%**	17.600[a]± 1.341	28.956 [b]± 1.429
***Ginkgo biloba* leaves 10%**	17.950[a]± 0.836	25.062 [c]± 0.881
***C. tora* seeds 5%**	17.970[a]± 0.330	28.972 [b]± 1.950

***C. tora* seeds 10%**	18.310[a]±0.466	23.762 [c]± 1.111

Hch diet: hypercholesterolemic diet. The data is presented as a mean ± SE
Values with unlike letters vs. other letters at P<0.05.

The results of *Ginkgo biloba* leaves and *Cassia tora* seeds on serum cholesterol and triglycerides are shown in Table (2). Gb leaves and C. tora seeds at the tested levels significantly (P<0.05) decreased the lipid parameters (TC and TG) in comparison to the +ve group at levels (5 and 10%). Rats fed Gb leaves or C. tora seeds at a 10% concentration show significantly lower mean values of (TC, and TG) than rats fed Gb leaves or C. tora seeds at a 5% concentration. There are no substantial triglyceride changes among Gb leaves or C. tora seeds 5% and 10%. The most remarkable improvement in serum cholesterol and triglyceride were found in the group that consumed Ginkgo biloba leaves at 10%.

In accordance with the findings of the current investigation, **Hussain *et al*., (2022)** found that Ginkgo significantly reduced TG and LDL-c levels while significantly raising HDL-c levels when compared to baseline values. **Mustafa and Hussein, (2023)** observed that Gb reduced cholesterol and triglyceride levels in the blood, decreased the level of LDL and improved the lipid profile. Also, **Wang *et al*., (2023)** demonstrated that blood lipid parameters and liver fat content could be dramatically improved by the use of GBE. Blood testing revealed improvements in the fat profile by the use of GB, including decreased triglycerides and cholesterol **(Banin *et al*., 2021)**. Furthermore, a study by **Wang *et al*., (2022)** demonstrated that *Ginkgo biloba* leaf extract taken orally is beneficial in reducing hypercholesterolemia and atherosclerosis.

Regarding Cassia tora, **Ye *et al*., (2021)** showed that cassia seed aqueous extract treatment significantly improved lipid distribution. **Kumar *et al*., (2017)** reported that the anti-dyslipidemic and antioxidant capabilities of C. tora extract have protective benefits against diabetic dyslipidemia and related consequences. **Awasthi *et al*., (2015)** showed that feeding with C. tora seed extract at a dose of 500 mg/kg b.w. had a significant lipid-lowering effect by reversing plasma levels of TC and

TG. Similar, **Tzeng *et al*., (2013)** showed that C. tora seed extract (CSEE) (300 mg/kg/day) decreased plasma levels of TC and TG in rats receiving the HFD. The hypolipidemic properties of *Ginkgo biloba* leaves and C. tora seed have been hypothesised to be caused by all or part of these bioactive substances.

Table (2): Effect of *Ginkgo biloba* leaves and *Cassia tora* seeds on serum Total cholesterol (TC) and Triglycerides (TG) in hypercholesterolemic rats

Parameters / Groups	TC	TG
	mg/dl	
Control (-ve)	85.400 [e] ± 1.949	46.200 [c] ± 4.024
Control (+ve) Hch diet	157.200 [a] ± 8.074	66.800 [a] ± 4.086
***Ginkgo biloba* leaves 5%**	116.000 [c] ± 3.162	53.800 [b] ± 3.420
***Ginkgo biloba* leaves 10%**	91.600 [d] ± 5.941	52.400 [b] ± 3.130
***C. tora* seeds 5%**	130.642 [b] ± 2.648	55.722 [b] ± 0.542
***C. tora* seeds 10%**	119.400 [c] ± 2.792	52.722 [b] ± 3.010

Hch diet: hypercholesterolemic diet. The data is presented as a mean ± SE.
Values with unlike letters vs. other letters at $P<0.05$.

The effect of *Ginkgo biloba* leaves and *Cassia tora* seeds on serum lipoproteins are shown in Table (3). Gb leaves and C. tora seeds at the levels (5 and 10 %) significantly ($P<0.05$) decreased LDL-c and VLDL-c while significantly increasing HDL-c in comparison to the +ve control group. The group that received 10% of *Ginkgo biloba* leaves showed the greatest improvement in lipid profiles. In accordance with the findings of the current investigation, **Awad *et al*., (2021)** administered Gb orally showed a significant reduction in TC, TG, and LDL while, increase in HDL. These results agreed with **Eisvand *et al*., (2020)** demonstrated that *G. biloba* controls the blood fatty acid profile by reducing LDL and TG levels and raising HDL levels. Because, it possesses antihyperlipidemic activities. Regarding Cassia tora, **Awasthi *et al*., (2015)** showed that feeding with 500 mg/kg of C. tora seed extract improved on HDL levels. **Tzeng *et al*., (2013)** showed that CSEE (300 mg/kg/day) decreased plasma levels of LDL and raised HDL levels in rats receiving an HFD.

Table (4) displays the effects of *Ginkgo biloba* leaves and *Cassia tora* seeds on liver functions. The positive control group's levels (+ve) of AST, ALT, and ALP were significantly higher (P<0.05) compared to the negative control group. The supplemented Gb leaves or C. tora L. seeds significantly (P<0.05) lowered the mean levels of liver functions when compared to the +ve control group. Additionally, there have been significant changes in the treated groups. Additionally, it was found that liver enzyme levels fell with increasing dosages of Gb leaves or C. tora seeds. Moreover, the group receiving 10% of *Ginkgo biloba* leaves was shown to have the most notable improvement in liver function.

Table (3): Effect of *Ginkgo biloba* leaves and *Cassia tora* seeds on serum lipoproteins in hypercholesterolemic rats

Parameters / Groups	HDL-c	LDL-c	VLDL-c
	mg/dl		
Control (-ve)	63.940 [a]± 2.000	12.220 [e]± 1.458	9.240 [c]± 0.804
Control (+ve) Hch diet	20.140 [d]± 2.010	123.700 [a]± 8.353	13.360 [a]±0.817
***Ginkgo biloba* leaves 5%**	53.340 [b]± 2.965	51.900 [c]± 0.696	10.760 [b]± 0.684
***Ginkgo biloba* leaves 10%**	60.000 [a]± 3.994	21.120 [d]± 2.682	10.480 [b]± 0.626
***C. tora* seeds 5%**	40.100 [c]± 4.792	79.397 [b]± 3.738	11.144 [b]± 0.108
***C. tora* seeds 10%**	55.540 [b]± 3.597	53.312 [c]± 1.339	10.544 [b]± 0.602

Hch diet: hypercholesterolemic diet. The data is presented as a mean ± SE
Values with unlike letters vs. other letters at P<0.05.

The results agreed with **Niveen *et al*., (2023)** and **Ibrahim *et al*., (2022)** indicated that administering *Ginkgo biloba* leaf powder to nephrotoxic rats reduced serum levels of AST and ALT in the three levels, 1, 2, and 4%. **Asiwe *et al*., (2022)** demonstrated that taking a *Ginkgo biloba* supplement enhanced kidney and liver function and had a protective effect. Similar to, **Gadalla *et al*., (2023)** demonstrated that supplementing with Gb significantly reduced blood ALT and AST levels after 8 weeks. The study supports the findings of **Awad *et al*., (2021)** showed that liver enzymes significantly decreased when Gb was given orally to diabetic rats. Regarding Cassia tora, **Kim & Park, (2022)** indicate that C. tora therapy has a strong therapeutic potential for liver injury. The study supports the findings of **Ye *et al*., (2021)** demonstrated that treatment with C. seed aqueous extract significantly improved liver indicators. CSEE targeting may be a promising strategy for the management of non-alcoholic fatty liver disease associated with obesity **(Tzeng *et al*., 2013)**.

Table (4): Effect of *Ginkgo biloba* leaves and *Cassia tora* seeds on liver functions in hypercholesterolemic rats

Parameters / Groups	AST	ALT	ALP
	L/l		
Control (-ve)	111.000 [f]± 4.123	32.400 [f]± 2.408	267.800 [f]± 11.009
Control (+ve) Hch diet	229.600 [a]± 9.939	179.800 [a]± 9.338	1030.00 [a]± 31.200
***Ginkgo biloba* leaves 5%**	163.400 [c]± 6.580	77.000 [c]± 2.645	720.200 [c]± 7.661
***Ginkgo biloba* leaves 10%**	134.800 [e]± 3.114	55.000 [e]± 6.164	422.00 [e]± 15.984
***C. tora* seeds 5%**	186.400 [b]± 6.877	99.200 [b]± 9.855	824.00 [b]± 15.109
***C. tora* seeds 10%**	144.800 [d]± 7.791	67.600 [d]± 4.560	619.600 [d]± 19.730

Hch diet: hypercholesterolemic diet. The data is presented as a mean ± SE
Values with unlike letters vs. other letters at P<0.05.

Table (5) shows the results of *Ginkgo biloba* leaves and *Cassia tora* seeds on the kidney functions of hypercholesterolemic rats. Serum urea, uric acid, and creatinine are all significantly (P<0.05) higher in the (+ve) group When compared to the (-ve) group. While supplementation with Gb leaves or C. tora seeds at levels 5 and 10% significantly reduced the level of renal functions in contrast to the (+ve) group. However, Gb leaves or C. tora seeds at 10% significantly reduced serum urea and uric acid compared to Gb leaves or C. tora seeds at 5%. There are no appreciable changes in blood urea or uric acid between Gb leaves and C. tora seeds at levels of 5% or 10%. The rats fed on 10% Gb leaves showed the most improvement in renal functioning. The results agreed with **Niveen *et al*., (2023)** and **Ibrahim *et al*., (2022)** showed that the powdered Gb leaves reduced serum levels of urea and creatinine relative to the nephrotoxicity rats control group. The study supports the findings of **Nnamdi Nwokoma *et al*., (2022)** suggests that Flavonoids in the supplement are thought to have protected the kidney from renal toxicity.

Awad *et al*., (2021) showed that GbE might control serum urea, and creatinine levels. Our findings are consistent with those made public by **Chang *et al*., (2021)**, who found that Gb Leaves improved kidney function.

Regarding Cassia tora, **Ye *et al*., (2021)** showed that cassia seed aqueous extract treatment significantly improved renal markers. **Park *et al*., (2019)** reported that the serum creatinine levels in the C. tora extract were significantly lower when compared to +ve group, and C. tora protects renal tissue from renal damage.

Table (5): Effect of *Ginkgo biloba* leaves and *Cassia tora* seeds on kidney functions in hypercholesterolemic rats

Groups \ Parameters	Urea Nitrogen	Uric Acid	Creatinine
	mg/dl		
Control (-ve)	53.600 [d]± 3.577	1.620 [e]± 0.178	0.444 [e]± 0.055
Control (+ve) Hch diet	80.800 [a]± 5.019	2.780 [a]± 0.164	0.986 [a]± 0.073
***Ginkgo biloba* leaves 5%**	63.000 [b]± 3.464	2.060 [bc]± 0.230	0.730 [c]± 0.029
***Ginkgo biloba* leaves 10%**	56.200 [c d]± 2.489	1.760 [de]± 0.114	0.652 [d]± 0.037
***C. tora* seeds 5%**	59.200 [b c]± 4.324	2.260 [b]± 0.151	0.806 [b]± 0.030
***C. tora* seeds 10%**	53.600 [d]± 3.577	1.880 [cd]± 0.078	0.724 [c]± 0.039

Hch diet: hypercholesterolemic diet. The data is presented as a mean ± SE
Values with unlike letters vs. other letters at P<0.05.

The results of Ginkgo biloba leaves and Cassia tora seeds on antioxidant enzymes are in Table (6). Gb leaves and C. tora seeds at the tested doses significantly (P<0.05) increased the antioxidant enzymes (SOD and CAT) and significantly reduced MDA compared to the (+ve) group. Rats fed on Gb leaves or C. tora seeds at 10% compared to Gb leaves or C. tora seeds at 5% showed a significant increase in the mean values of (SOD and CAT) and a significant decrease in serum MDA. There are no substantial SOD changes among C. tora seeds 5% and 10%. The group that received 10% of Ginkgo biloba leaves showed the greatest improvement in antioxidant enzymes. According to **Moura *et al* (2015)**, Plants with flavonoids and polyphenols have been shown to have antioxidant activity, which may have a protective effect. The antioxidant defence system, which is made up of the enzymes CAT and

SOD, often eliminates excess free radicals produced inside a biological system **(Farombi *et al*., 2016)**.

The results agreed with **Gadalla *et al*., (2023)** who observed that the value of MDA was much lower in the aqueous extract of G. biloba leaf (GbE) group compared to the CCl4 group, and the results of the rats receiving (GbE) tended to be similar to the control value. GbE as a mechanism to reduce acute liver damage through the removal of oxidative free radicals, prevention of lipid peroxidation, and antioxidant activity **(Gadalla *et al*., 2023)**. The study supports the results of **Niveen *et al*., (2023)** and **Ibrahim *et al*., (2022)** illustrated that GB leaf powder significantly ($P<0.05$) lowered the serum concentration of MDA at the three levels of 1, 2, and 4% in nephrotoxic rats. Similar to, **Asiwe *et al*., (2022)** they showed that a Gb supplement (GBS) indicated a protective effect and enhanced anti-oxidant enzyme activity (CAT, SOD, and MDA). These results were similar to those obtained by **Mustafa and Hussein, (2023)** and **Chen *et al*., (2019)** indicate that Gb extract acted by raising the expression of SOD, CAT, or by lowering both ROS and MDA. **Yan *et al*., (2020)** indicated that GbE enhanced antioxidant enzyme activities and reduced lipid peroxidation.

Regarding Cassia tora, these findings were consistent with those made by **Bhargava *et al*., (2023)** showed that treatment of C. tora significantly declined the MDA levels and augmented the levels of CAT and SOD. Similar, **Anyebe *et al*., (2021)**, who found that mice treated with C. tora increased SOD levels, CAT activities while decreased MDA levels compared to untreated mice. According to **Moura *et al*.,(2015)**, showed that Alkaloids, flavonoids, triterpenoids, and steroids are some phytochemicals found in C. tora that may prevent or decrease oxidative damage. The results agreed with **Awasthi *et al*., (2015)** C. tora may have anti-hypercolestremic qualities and contribute significantly to oxidative stress, because of its high antioxidant activity. The study supports the findings of **Ye *et al*., (2021)** showed that cassia seed aqueous extract treatment significantly improved oxidative status. Also, **Park *et al*.,**

(2019) reported that C. tora extract significantly enhanced the activities of oxidation-related markers through increase of CAT and SOD levels.

Conclusions:

In the present study, we demonstrated that *Ginkgo biloba* Leaves or *Cassia tora* seeds supplementation can lower the levels of LDL-cholesterol in the blood during hypercholesterolemia. By encouraging both the absorption of LDL-cholesterol into tissue and cholesterol excretion, as well as blocking cholesterol synthesis. These results indicate that Gb Leaves and C. tora seeds have a preventative effect against the development of atherosclerosis and hypercholesterolemia at doses of 5 and 10 % and improve kidney, liver enzymes and antioxidant enzymes. So, *Ginkgo biloba* Leaves and *Cassia tora* seeds may be recommended for hypercholesterolemic patients.

Table (6): Effect of *Ginkgo biloba* leaves and *Cassia tora* seeds on antioxidants enzymes in hypercholesterolemic rats

Parameters / Groups	MDA nmol/ml	SOD U/ml	CAT U/L
Control (-ve)	1.546 [e] ± 0.654	176.00 [a] ± 5.196	331.00 [a] ± 8.717
Control (+ve) Hch diet	29.891 [a] ± 1.517	116.00 [e] ± 4.582	82.667 [f] ± 6.658
***Ginkgo biloba* leaves 5%**	9.203 [c] ± 0.262	125.00 [d] ± 1.732	130.333 [e] ± 3.214
***Ginkgo biloba* leaves 10%**	2.890 [e] ± 0.378	145.667 [b] ± 4.163	241.666 [b] ± 6.429
***C. tora* seeds 5%**	12.200 [b] ± 0.720	130.00 [cd] ± 1.702	162.656 [d] ± 3.468
***C. tora* seeds 10%**	5.206 [d] ± 0.749	134.00 [c] ± 1.730	191.667 [c] ± 7.094

Hch diet: hypercholesterolemic diet. The data is presented as a mean ± SE
Values with unlike letters vs. other letters at P<0.05.

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